

- 2 -

Figure 1 is a schematic representation of the oligonucleotide maps of the recombinant viruses.

Bicon
Figure 2 (SEQ.ID.NO: 1) is a schematic and nucleic acid sequence of the fgl2 gene.

Figures 3A and 3B are bar graphs showing induction of fgl2 in the presence of various viruses.--

Please replace the paragraphs beginning at page 21, line 22 to page 24, line 4 with the following rewritten paragraphs:

Bz
-Expression of fgl2 was detected by RT-PCR. Freshly isolated macrophages, at a multiplicity of infection (MOI) of 2.5, were infected with different strains of viruses for 6 hours. 1×10^7 macrophages were pelleted in 1.5 ml Eppendorf tube and total cellular RNA was isolated by 8M acid-guanidium hydrochloride extraction in a modified procedure as previously described (Evans and Kamdar, 1990). The quantity and quality of RNA was examined by spectrophotometry and on a 1% analytical agarose gel containing formaldehyde. RNA (5mg) was reverse transcribed using Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLVRT) in 20 ml reactions, as recommended by manufacturer. PCR was then performed in 50 ml reactions using 1ml portion of cDNA and the primers fgl2-318 (TGC CCA CGC TGA CCA TCC A) (SEQ.ID.NO: 2) corresponding to nucleotide 318 to 336 of Balb/c fgl2 cDNA (M 15761) and FGL2-1224 (GAG ACA ACG ATC GGT ACC CCT) (SEQ.ID.NO: 3) corresponding to nucleotides 1224 -1244 of Balb/c fgl2 cDNA. (M16238), which yield a 906 bp band in 1% agarose DNA gel. Amplification products were not obtained when reverse transcriptase was omitted (data not shown). RT-PCR for GAPDH was also set up as an internal control to assess the quality of first strand synthesis.

Creation of N gene and fgl2 promoter constructs

- 3 -

Restriction enzymes used to create constructs were obtained from GIBCO BRL, Life Technologies, USA. All plasmids were purified using Qiagen Maxiprep Kits, and grown in DH5 *E. coli* bacteria (GIBCO BRL).

MHV-2 and A59 are different sizes. The entire N gene coding regions and 3' UTRs of MHV-A59, MHV-2, ML3 and ML11 were amplified by RT-PCR. RNA was originally extracted from infected macrophages. The sense primer ACG ATG TCT TTT GTT CCT GGG (SEQ.ID.NO: 4) was phosphorylated chemically at position 1 to achieve directed insertion and ligation of PCR products to its vector; the anti sense primer at position 1654 TTT TTT TTT GTG ATT CTT CCA (SEQ.ID.NO: 5) had a poly T group to match the poly A tail at the 3' end of nucleocapsid genomic RNA. The N gene fragments were subcloned into the 5.0 Kb expression vector pCR 3.1 (Invitrogen), under the control of the CMV promoter and bovine growth hormone 3'-processing signals. External restriction endonuclease Hind III and Pst I and internal restriction endonuclease Eco RI, Eco RV were used to analysis the size and orientation of N gene insert in recombinant plasmid constructs.

Luciferase Reporter Constructs: A 1.3 kb DNA fragment flanking the 5' end of mouse *fgl2* was released by restriction digestion with EcoRV and Sal I from a subclone pBluescript-m166 (pm166) of mouse genomic P1 plasmid (Genome System Inc.) which contains the entire mouse *fgl2* gene. This fragment was sequenced by cycle sequencing on an automated DNA sequencer (Model 377, Applied Biosystems) using dideoxy dye terminator chemistry. This sequence has been deposited into Genbank with the Accession number AF025817 (Figure 2) (Koyama et al., 1987). This 1.3 kb fragment was inserted into Sma I and Xho I sites of the pGL2-basic luciferase reporter vector (Promega) to form p*fgl2*(-1328)LUC. 5' deletion constructs of *fgl2* promoter were made by first amplifying the specific fragment using pm166 as template and then cloned into PCR 2.1 cloning vector (Stratagene) and resubcloned into pGL2-basic plasmid at Xho I and Hind III sites. The

- 4 -

reverse primer (GCC ACA ACC AAC CAG GAA G) (SEQ.ID.NO: 6) was used to make all deletion constructs by-PCR amplification. The upstream primers used were: GAG CTG AGT GAT GGG GAA GGA (SEQ.ID.NO: 7) for pfgl2(-693)LUC, CCA CTG ACG ATT ACA TAG CC (SEQ.ID.NO: 8) for pfgl2(-625)LUC, GGA CCT TTG TTC TGA TTA GGG GC (SEQ.ID.NO: 9) for pfgl2(-511)LUC, CGC AGA CAT TTA GAC GTT CC (SEQ.ID.NO: 10) for pfgl2(-372)LUC, and GGG CAC TGG TAT TAC AAC TGT (SEQ.ID.NO: 11) for pfgl2(-306)LUC. All promoter-luciferase report constructs were sequenced to confirm the orientation and to verify the sequence. Positive control, pGL2 Control plasmid with SV-40 promoter, and RSV b-gal vector were from Promega. A 2Kb tissue factor promoter construct pTF(-2Kb)LUC was a kind gift of Dr. Nigel Mackman (Mackman et al., 1990).

h2
N gene Sequence of different virus strains

The N gene sequence of multiple clones for each strain of virus was determined using primer-directed strategies by cycle sequencing on an automated DNA sequencer, using the ABI PRISMTM dRhodamine Terminator Cycle Sequencing Reading Reaction Kit (Model 377, PE Applied Biosystems). The T7 primer and pCR3.1 reverse primer were used for 5' to 3' and 3' to 5' sequence, respectively. A new primer CTC AGG GCT TTT ATG TTG AAG (SEQ.ID.NO: 12) (MHV-ND557) at position 557 was also designed based on the outcome of sequencing and the published cDNA sequence of MHV-A59 to complete the sequencing. Extension products were purified by ethanol/sodium acetate precipitation. Samples were subjected to electrophoresis on the ABI PRISM 310. The sequence was analyzed using the DNAsis for windows, sequence analysis software (Hitachi Software Engineering America Ltd, San Bruno, CA, USA).—

Please replace the paragraph beginning at page 29, line 5 with the following rewritten paragraph:

--For the LF-A1 site (-338/-319 of fgl-2 promoter region):